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ANIMAL AND PLANT HEALTH INSPECTION SERVICE
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SAM - 102

9 CFR 113.144	Revised January 1982	Parainfluenza-3
Standard Requirement	Supersedes April 15, 1968	Agent

SUPPLEMENTAL ASSAY METHOD

FOR

TITRATION OF PARAINFLUENZA-3 VIRUS IN VACCINES

A. SUMMARY

This is an *in vitro* assay method which employs a cell culture system and uses cytopathic effect (CPE) and/or hemadsorption (Had) of guinea pig erythrocytes to determine the parainfluenza-3 viral content of vaccines.

B. MATERIALS

1. Cell Culture and Preparation

Tissue culture multi-well plates with covers (24 flat bottom wells - 1.7 X 1.6 cm) containing monolayers of secondary or tertiary bovine embryonic kidney (BEK) cells are used to titrate the vaccine virus.

a. Secondary or tertiary cells are removed from the growth containers by using a Trypsin-Versene solution (T-V)(Appendix 1).

b. The cells are counted and diluted from 200,000 to 300,000 cells/ml with growth medium (Appendix 2) and 0.5 ml planted into each well.

c. The cell cultures are incubated at 35 to 37 C in an atmosphere of 3 to 5% carbon dioxide and high humidity for 2 or 3 days or until the cell monolayers are at least 75% confluent.

2. Reference or Control Virus

Veterinary Biologics' reference PI-3 virus is titrated as a control for the cell system.

3. Growth Medium and Diluent

Minimum essential medium (MEM)(Appendix 2) is used for growth of cells. MEM (without serum) is used to make dilutions of the vaccine and control viruses.

4. Maintenance Medium

Same as growth medium, except serum may be reduced to a 5% level*

5. Antisera

IBR and BVD specific immune sera are used to neutralize these viruses.

6. Guinea pig erythrocytes for HAd test (Appendix 3).

C. METHOD

1. The vaccine to be assayed for PI-3 virus titer is rehydrated with the accompanying diluent.

2. In order to determine the PI-3 virus titer in combination vaccine products, it is necessary to neutralize the Infectious Bovine Rhinotracheitis (IBR) and Bovine Viral Diarrhea (BVD) viruses with specific immune serum. This leaves the PI-3 virus fraction to be titrated.

   a. IBR/PI-3 Vaccine

      (1) The rehydrated vaccine is diluted 1:5 (1+4) with diluent.

      (2) Equal volumes of the 1:5 dilution of vaccine and IBR specific immune serum are mixed.

      (3) This mixture is held at room temperature for 45 minutes to neutralize the IBR virus.

* The serum level may be varied, depending upon the condition of the cells.

(4) The mixture constitutes a 10^{-1} dilution of the vaccine containing the PI-3 virus to be titrated.

b. IBR/BVD/PI-3 Vaccine

(1) The rehydrated vaccine is diluted 1:5 (1+4) with diluent.

(2) One ml of the 1:5 dilution of vaccine is mixed with 0.5 ml IBR specific immune serum and 0.5 ml BVD specific immune serum.

(3) This mixture is held at room temperature for 45 minutes to neutralize the IBR and BVD viruses.

(4) The mixture constitutes a 10^{-1} dilution of the vaccine containing the PI-3 virus to be titrated.

3. Serial 10-fold dilutions are made from the 10^{-1} dilution of the PI-3 vaccine virus and the reference virus using diluent without serum.

4. Just before inoculation of cells, the growth medium is removed from the 24-well plate (by aspiration) and discarded.

5. Two-tenths ml of each dilution of test vaccine virus and the reference virus are inoculated onto 5 cell culture wells. After inoculation, 0.8 ml maintenance medium is added to each well. These plates are incubated at 35 to 36 C in an atmosphere of 3 to 5% carbon dioxide and high humidity for 4 or 5 days.

6. After 4 or 5 days the cells can be examined for cytopathology typical for PI-3 virus.

   The cells also can be tested for hemadsorption of guinea pig erythrocytes as follows:

   a. Fluid is removed from the cell plate wells and is replaced with 0.5 ml of a 0.5% washed erythrocyte suspension (Appendix 3).

   b. The plates are left at refrigerator temperature for 15 or 20 minutes.

   c. Fluid is removed from the wells and the cell layers are washed 3 times with phosphate buffered saline and examined for hemadsorption. The adsorbed erythrocytes will not wash from virus laden cells. Readings should be completed within 20 minutes.

   d. The number of wells showing hemadsorption are recorded and the 50% endpoint is calculated by the Reed-Muench or Spearman-Kärber method.

D. APPENDIX

1. T-V Solution:

NaCl	0.8 %
KCl	0.04 %

$NaHCO_3$	0.058%
Trypsin (Difco 1:250) BPL * treated	0.05 %
Versine (Disodium Salt)	0.02 %
Dextrose	0.1 %
Distilled H_2O q.s. ad	100.0 %

Sterile filtered and stored frozen.

2. Growth Medium:

Minimum Essential Medium (MEM)

MEM (Eagle) with Earles' Salts ** q.s. ad	100.0 %
L-Glutamine	1.0 %
Edamin 0.5%	0.5 %
Goat or fetal calf serum heat inactivated	10.0 %

* B-Propiolactone

** Available from Grand Island Biological Company, Inc. No endorsement expressed or implied

Antibiotics:

Gentamicin . . . . . 50 mcg per ml

Amphotericin B . . . . . 2.5 mcg per ml

3. Guinea Pig Erythrocytes (rbc):

Blood is collected aseptically from healthy adult guinea pigs and is added to an equal amount of sterile Alsever's solution.

Alsever's solution:

Dextrose . . . . . 2.05 %

Sodium Citrate . . . . . 0.8 %

NaCl . . . . . 0.42 %

Citric Acid 0.055%

Distilled H_2O q.s. ad . . . . . 100.0 %

The erythrocytes are washed 3 times in Alsever's solution and sedimented by centrifugation (1,000 to 1,200 rpm for 15 minutes).

The cells can be stored as a 50% suspension at 4 C.

For testing, the erythrocytes are used as a 0.5% suspension in phosphate buffered saline.